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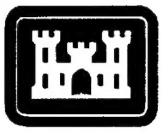

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Aquatic Plant Control Research Program

Efficacy of AVAST!® Fluridone Formulation Against Eurasian Watermilfoil and Nontarget Submersed Plants

Angela G. Poovey, John G. Skogerboe, and Kurt D. Getsinger June 2004

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Final report

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ABSTRACT: Experiments were conducted in a laboratory and an outdoor mesocosm system to evaluate the liquid AVAST!® fluridone formulation for control of Eurasian watermilfoil (*Myriophyllum spicatum*). In addition, physiological assays were used to monitor plant injury during herbicide exposure. Eurasian watermilfoil was planted in 52-L aquaria, grown to precanopy condition (21 days), then dosed with 0, 10, 20, 40, and 80 μg L^{-1} active ingredient (ai) fluridone for exposure times of 45 and 90 days. Elevated phytoene concentrations indicated herbicide exposure in all treated plants at 7 days after treatment (DAT). Visually, plants did not manifest symptoms of injury from fluridone until 14 DAT. β-carotene concentrations suggested that fluridone disrupted photosynthesis as soon as 7 DAT. Both formulations were effective in controlling Eurasian watermilfoil. Biomass decreased by 90 percent at all application rates following the 45-day exposure and decreased by 99 percent following the 90-day exposure time. No significant differences occurred between application rates at either exposure time. Based on these results, rates of 6, 12, and 24 μg ai L^{-1} fluridone were applied to Eurasian watermilfoil and four nontarget submersed aquatic species in an outdoor mesocosm system for a 56-day exposure time. Gradual herbicide dissipation in the mesocosms resulted in half-lives ranging from 23 to 24 days. Again, the AVAST!® fluridone formulation was effective in controlling Eurasian watermilfoil. Biomass was reduced by >85 percent at all doses compared to the untreated reference. The AVAST! formulation did not significantly decrease biomass from wild celery (*Vallisneria americana*) and Illinois pondweed (*Potamogeton illinoensis*) at any application rate. Biomass levels from elodea (*Elodea canadensis*) and sago pondweed (*Stuckenia pectinata*) were reduced at 12 and 24 μg ai L^{-1}. Symptoms of injury were evident in these plants by depressed β-carotene pigment concentrations at 28 DAT; however, β-carotene levels increased in sago pondweed by 56 DAT, suggesting recovery.

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Preface

The work reported herein was conducted as part of the Aquatic Plant Control Research Program (APCRP), Work Unit Number 74001. The APCRP is sponsored by Headquarters, U.S. Army Corps of Engineers (HQUSACE), and is assigned to the U.S. Army Engineer Research and Development Center (ERDC) under the purview of the Environmental Laboratory (EL), Vicksburg, MS. Funding was provided under Department of the Army Appropriation 96X3122, Construction General. Mr. Robert C. Gunkel, Jr., EL, ERDC, was Program Manager for the APCRP. Program Monitor during this study was Mr. Timothy R. Toplisek, HQUSACE.

The Principal Investigator of this work was Dr. Kurt D. Getsinger, Environmental Processes Branch (EPB), Environmental Processes and Engineering Division (EPED), EL, ERDC. This work was conducted and report prepared by Ms. Angela G. Poovey, Mr. John G. Skogerboe, and Dr. Getsinger, EPB.

Technical reviews of this report were provided by Ms. Lee Ann Glomski, Purdue University, West Lafayette, IN; Dr. John D. Madsen, Mississippi State University; and Mr. Craig Smith, Griffin LLC, Valdosta, GA. The authors wish to thank Ms. Anne Stewart, CSC, Vicksburg, MS, for plant tissue analyses and Mr. David Honnell, North Texas University, Denton, TX, for water residue analyses. Technical assistance was provided by Ms. Toni Pennington, Mr. Stephen George, and Mr. Lee Davis. The cooperation of Griffin LLC for providing herbicide and additional support for this project through the Aquatic Ecosystem Research Foundation is greatly appreciated.

This work was performed under the general supervision of Dr. Beth Fleming, Acting Director, EL; Dr. Richard E. Price, Chief, EPED; and Dr. Terrence M. Sobecki, Chief, EPB.

COL James R. Rowan, EN, was Commander and Executive Director of ERDC. Dr. James R. Houston was Director.

1 Introduction

Background

The aquatic herbicide fluridone (1-methyl-3-phenyl-5-[3-(trifluromethyl) phenyl]-4(1*H*)-pyridinone) has proved effective in controlling the exotic submersed macrophyte Eurasian watermilfoil (*Myriophyllum spicatum* L.; "milfoil"). Small-scale concentration/exposure time (CET) studies have demonstrated that doses of 5 to 40 µg L^{-1} active ingredient (ai) fluridone for exposure times of 60 to 90 days significantly reduce milfoil shoot biomass (Netherland et al. 1993, Netherland and Getsinger 1995a, 1995b). These rates are 75 to 95 percent below the maximum label rate of 150 µg ai L^{-1}. Moreover, meosocom evaluations have demonstrated how low rates of fluridone selectively controlled milfoil without harming native aquatic plants (Netherland et al. 1997, Nelson et al. 1998).

There is some debate among lake and fisheries managers about the selective plant control abilities of fluridone, especially when used in a whole-lake treatment scenario. Although cover and diversity of native species have typically recovered by 1 to 3 years post-treatment following whole-lake fluridone applications, even at rates >20 µg ai L^{-1} (Getsinger 1993, Smith and Pullman 1997), much of the concern has focused on potential impacts to game fish populations following the removal of a portion of vegetation throughout the lake in the year of treatment. Because of these concerns, the "booster" or "bump" fluridone treatment strategy has been implemented in some northern tier lakes being treated for milfoil control. This treatment strategy utilizes a low initial dose application of 5 to 10 µg ai L^{-1} fluridone followed by a booster or bump application 2 to 3 weeks following the initial treatment. Field evaluations using the booster fluridone treatment strategy have resulted in excellent milfoil control while many of the native plant populations were maintained in the year of treatment and beyond in Michigan (Getsinger et al. 2001) and Vermont lakes (Getsinger et al. 2002b).

These and other investigations used the formulation of Sonar® AS (aqueous suspension) because it was the only formulation of fluridone available at the time. Recently, another fluridone aqueous suspension formulation has been manufactured and distributed under the trade name AVAST!®. To evaluate the efficacy of the AVAST! formulation of fluridone against milfoil, a CET study in a controlled-environment growth chamber and a selectivity study in an outdoor mesocosm system were conducted.

As a part of these studies, physiological assays related to the fluridone mode of action were also conducted. Such assays have the advantage of revealing the onset and duration of a herbicide effect before visual symptoms occur (Sprecher and Netherland 1995). The herbicide fluridone interrupts carotene biosynthesis in newly emerging tissue by blocking phytoene desaturase, an enzyme necessary for production of the intermediate, phytofluene (Bartels and Watson 1978; Sandmann and Böger 1983). Because phytofluene is not produced, phytoene, another intermediate pigment, accumulates, and the carotenoids, α-carotene and β-carotene, are not synthesized (Figure 1). Carotenoids are yellow pigments that help plants photosynthesize, and protect the chlorophyll pigments from photoxidation under stressful photosynthetic conditions. Damaged chlorophyll limits the photosynthetic process, and plants eventually die.

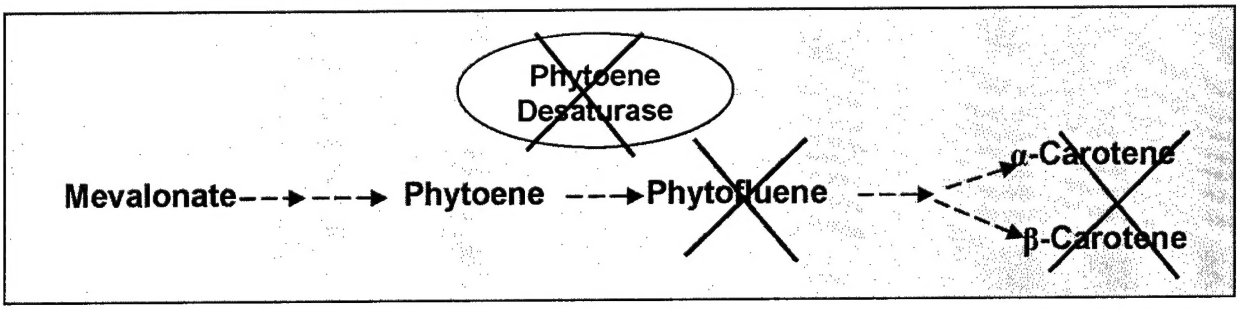

Figure 1. Fluridone mode of action in the carotenoid biosynthetic pathway of newly emerging tissue

Visual symptoms of fluridone exposure are bleaching of plant apices (chlorosis) as chlorophyll is destroyed via photooxidation (Bartels and Watson 1978). Plant apices may also appear light pink or purple when the duration or dose of fluridone increases as the anthocyanin pigments are unmasked after chlorophyll photooxidation (Doong et al. 1993). Previous mature stems remain green and continue to photosynthesize.

In the past, monitoring chlorophyll has been a physiological assay used in small-scale fluridone research studies to track and predict control of milfoil (Netherland et al. 1993; Netherland and Getsinger 1995a, 1995b). Because the degree of chlorosis produced by fluridone in emerging tissues has been shown to be dose proportional in several species, chlorophyll data documented plant injury and, in conjunction with shoot biomass weight, determined efficacy. Recently, Sprecher et al. (1998) demonstrated how elevated concentrations of the colorless pigment phytoene in aquatic plant tissues were unique to fluridone exposure.

Although all plants have trace amounts of phytoene, when exposed to fluridone, most plants rapidly accumulate significant amounts of phytoene, which can be easily measured using tissue extracts in an ultraviolet spectrophotometer. Relatively high phytoene concentrations indicate fluridone exposure, while low β-carotene concentrations may correspond to a shoot biomass reduction in an aquatic plant species (Doong et al. 1993, Sprecher et al. 1998).

Objectives

To evaluate the efficacy of the AVAST! formulation of fluridone against milfoil, a small-scale CET study in a controlled environment growth chamber, and a mid-scale selectivity study using milfoil and four nontarget submersed species in an outdoor mesocosm system, were conducted. The objectives of these studies were to:

a. Determine the effectiveness of AVAST! against the target plant, milfoil, under a range of CET relationships.

b. Determine the species selective potential of AVAST! when used at low doses.

c. Confirm the use of previously developed assays to monitor plant injury when exposed to AVAST!.

2 Concentration/Exposure Time Study

Methods and Materials

In this small-scale study, plants were grown in 42 vertical aquaria (52 L) located in a walk-in controlled environment growth chamber (58 m^2). Environmental conditions were selected to provide ideal requirements for submersed plant growth with light intensity of 520 ± 50 µmol m^{-2} sec^{-1}, temperature of 20 ± 3 °C, and photoperiod of 14 hr:10 hr light:dark cycle. Lighting was provided by a combination of 400-watt high-pressure sodium and metal halide bulbs. Ultraviolet (UV) radiation was filtered by glass plates situated underneath the light bulbs. This lighting scheme eliminated fluridone degradation by UV wavelengths.

Four healthy apical cuttings (10 cm) of milfoil, obtained from Suwannee Laboratories (Lake City, FL), were planted in 300-ml glass beakers that contained sediment from Brown's Lake, Vicksburg, MS, which had been amended with 150 g L^{-1} ammonium chloride and 1 g L^{-1} slow-release fertilizer Osmocote® 15:15:15. A 1-cm layer of silica sand was added to the sediment surface to prevent suspension of sediment particles in the water column. Ten beakers were placed in each aquarium. Aquaria were filled with a culture solution recommended for growth of submersed aquatic macrophytes (Smart and Barko 1984). Plants grew for 21 days. Pretreatment biomass was estimated by removing plant shoots from two beakers in each aquarium. Shoots were dried for 48 hr then weighed to obtain a mean dry weight. Pretreatment shoot biomass (±1 SE) was 1.73 ± 0.13 g, which approximated early season biomass (155 g DW m^2; Grace and Wetzel 1978).

A stock solution of the AVAST! aqueous suspension formulation was prepared as 1 mg L^{-1}. From that stock, four rates, 10, 20, 40, and 80 µg ai L^{-1}, were applied to aquaria for static exposure times of 45 and 90 days. Untreated reference aquaria were included to assess plant growth and physiology in the absence of herbicide exposure.

Plant physiological status during treatment was monitored through measurement of plant pigments phytoene, β-carotene, and total chlorophyll. Apical plant stems were collected from each treatment at 7, 30, and 45 days after treatment (DAT), then analyzed. Sprecher et al. (1998) developed analytical

procedures for characterizing phytoene and β-carotene concentrations in fluridone-treated aquatic plants. Approximately 5 cm fresh apical shoot tissue from collected plant samples was weighed (0.25 to 0.5 g), then mechanically homogenized in 5 mL of a freshly made solution of 6 percent (w/v) KOH in MeOH. Tubes were capped and centrifuged for 5 min at 3000 rpm. The supernatant was decanted into a fresh tube containing 2 mL of light petrol (petroleum benzin, #85100, b.p. 80-110 °C, Fluka, Ronkonkoma, NY), and shaken vigorously. After separation of the epiphase (1 min), an aliquot was transferred to a 1.5-mL disposable UV semimicro cuvette (methacrylate, Dynalon, Rochester, NY) using a glass transfer pipette. Samples then were covered to avoid light. After the solution completely cleared (30 min), samples were read using a spectrophotometer (Beckman, DU-640, Fullerton, CA) Sample absorbance was read at 287 nm for phytoene and at 445 nm for β-carotene. Pigment concentrations were calculated using the following equation:

$$\text{Pigment concentration } (\mu g\ g^{-1}) = ([(A/E) * 2\ mL/100\ mL]/g\ FW) * 10^6$$

where

A = absorbance (287 nm for phytoene or 445 nm for β-carotene)

E = extinction coefficient (1108 for phytoene or 2500 for β-carotene)

$g\ FW$ = grams fresh weight of each sample

The analytical procedure for total chlorophyll concentration used was summarized by Hiscox and Israelstam (1979). Approximately 3 cm fresh apical shoot tissue from collected plant samples was weighed (0.1 to 0.3 g) and placed in 10 mL of dimethyl sulfoxide (DMSO). To extract chlorophyll into the DMSO, test tubes were placed in a water bath for 6 hr at 65 °C. After removing tubes from the water bath, a 3-mL aliquot was transferred to a disposable UV semimicro cuvette (methacrylate, Dynalon, Rochester, NY). Using a spectrophotometer (Beckman, DU-640, Fullerton, CA) sample absorbance was read at 645 nm and 663 nm. Samples with absorbance values greater than 1.2 were diluted 1:1 with DMSO to obtain a readable value. Pigment concentrations were calculated using the following equations (Arnon 1949):

$$\text{chlorophyll } a\ (mg\ g^{-1}) = ([(0.0127 * A_{663}) - 0.00269 * A_{645})] * 10\ mL)/g\ FW$$
$$\text{chlorophyll } b\ (mg\ g^{-1}) = ([(0.0229 * A_{645}) - (0.00468 * A_{663})] * 10\ mL)/g\ FW$$

where

A_{645} = absorbance at 645 nm

A_{663} = absorbance at 663 nm

$g\ FW$ = grams fresh weight of each sample

Total chlorophyll concentration equals the sum of chlorophyll *a* and chlorophyll *b* concentrations.

Herbicide efficacy was determined through the measurement shoot biomass dry weight. Four beakers were harvested after each exposure time, 45 and 90 DAT. Shoots were clipped at the sediment surface, oven dried at 70 °C for 48 hr, and then weighed.

Each treatment was replicated three times. Because values of zeros were present, biomass data were log(n+1) transformed to normalize data, then subjected to a one-way analysis of variance (ANOVA) to test for herbicide concentration effects at each exposure time. If pigment data did not meet the assumptions of normality and equal variance, data were log transformed, then analyzed using a one-way ANOVA at each sample date. If significant differences were detected for each ANOVA, means were separated using the Tukey test ($p \leq 0.05$).

Results and Discussion

All AVAST! treatments significantly reduced shoot biomass compared to the untreated reference for both exposure times (Figure 2). Biomass decreased by 90 percent for the 45-day exposure and 99 percent for the 90-day exposure. Biomass from both treatments were decayed stems and fragments. There were no significant differences between AVAST! rates at each harvest indicating that the lowest rate of 10 µg ai L^{-1} was as effective as the highest one, 80 µg ai L^{-1}. Netherland and Getsinger (1993) also reported no differences between Sonar® AS treatments of 12 and 24 µg ai L^{-1} after a 90-day static exposure in which both treatments decreased milfoil biomass by 99 percent. Adequate exposure time is essential for milfoil control. Another laboratory study showed that an initial treatment rate of 25 µg ai L^{-1} for a 28-day half-life with threshold concentrations of 3 µg ai L^{-1} still present in the water column at 105 DAT controlled milfoil by >90 percent, whereas an initial rate of 100 µg ai L^{-1} with a 10-day half-life with no fluridone in the water column by 60 DAT provided no control of milfoil (Netherland and Getsinger 1995b).

Phytoene concentrations ranged from 75.7 to 104 µg g FW^{-1}, indicating fluridone exposure in all treated milfoil plants at 7 DAT (Table 1). Elevated phytoene concentrations have been observed at 3 DAT for fluridone rates ranging from 1.0 to 2.0 µg ai L^{-1} (Sprecher et al. 1998) and 2 DAT for rates ranging from 5 to 10 µg ai L^{-1} (authors' unpublished data). Reference plants had phytoene levels of 14.9 µg g FW^{-1}, which correspond to those reported in another laboratory study (Sprecher et al. 1998). Only the 80 µg ai L^{-1} treatment visually exhibited chlorotic stems and apices at this time. By 14 DAT, the other treatments also manifested symptoms of herbicide injury, such as chlorotic purple apices on new growth.

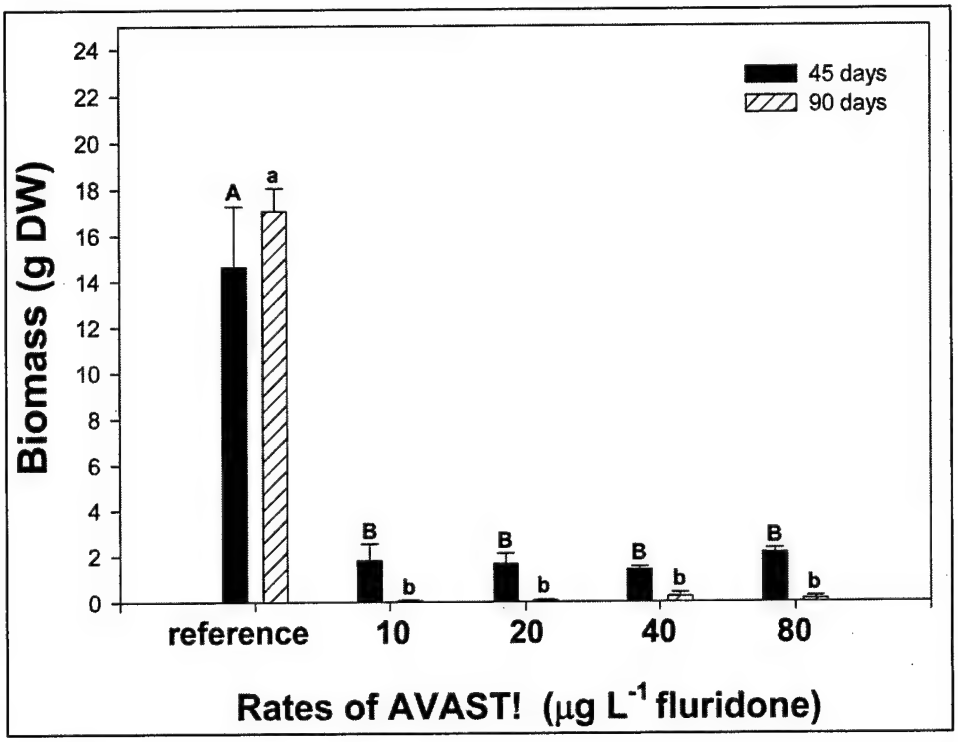

Figure 2. Milfoil shoot biomass (g DW) harvested after 45- and 90-day exposure to the AVAST! formulation of fluridone. Rates tested were 10, 20, 40, and 80 µg ai L^{-1}. Means are ±1 SE (n = 3). Capital letters denote significant differences between treatments after 45 days and small case letters denote significant differences between treatments after 90 days (Tukey test, $p \leq 0.05$).

As exposure to fluridone continued over time, all rates except the 80 µg ai L^{-1} at 30 DAT and 40 µg ai L^{-1}at 45 DAT maintained phytoene levels of >90 µg g FW^{-1}. Phytoene levels in the 80 µg ai L^{-1} treatment dropped from 75.7 µg g FW^{-1} at 7 DAT to 52.0 µg g FW^{-1} at 30 DAT. Phytoene accumulation decreased as the plant's physiological processes declined, evident by the very low β-carotene levels. These β-carotene levels represented an 87 percent decrease from the reference (Table 1). At 45 DAT, phyotene levels for the 40 µg ai L^{-1} treatment dropped to 52.6 µg g FW^{-1} and β-carotene levels were 1.63µg g FW^{-1}.

β-carotene concentrations for treated milfoil plants ranged from 3.25 to 14.5 µg g FW^{-1} at 7 and 30 DAT, which was 87 to 51 percent less than the reference plants (Table 1). The β-carotene data suggest that fluridone disrupted photosynthesis in these milfoil plants as soon as 7 DAT. Chlorophyll concentrations, however, showed that these plants were still photosynthesizing (Table 1). If fluridone were removed after 7 or 30 DAT, it is likely that plants treated with 10 to 40 µg ai L^{-1} would recover as shown in previous work (Netherland et al. 1993, Netherland and Getsinger 1995b, Sprecher et al. 1998). Recovery of milfoil treated with 10 to 20 µg ai L^{-1} after a 60-day exposure has also been reported (Netherland et al. 1993, Netherland and Getsinger 1995b). However, in this study, there were no intact plant apices at 60 DAT, as milfoil plants were dying.

Table 1
Pigment Concentrations in Milfoil Shoots 7, 30, and 45 DAT with the AVAST! Fluridone Formulation in a Concentration/Exposure Time Study[1]

Fluridone µg ai L^{-1}	Phytoene, µg g^{-1} FW			β-carotene, µg g^{-1} FW			Chlorophyll, mg g^{-1} FW		
	7 DAT	30 DAT	45 DAT	7 DAT	30 DAT	45 DAT	7 DAT	30 DAT	45 DAT
0	14.9 ± 0.18b	24.8 ± 1.47c	14.3 ± 2.92b	28.5 ± 2.60a	25.8 ± 1.06a	28.1 ± 3.46a	1.39 ± 0.03a	1.71 ± 0.28a	1.52 ± 0.14a
10	81.2 ± 4.55a	124 ± 4.28a	103 ± 6.47a	14.1 ± 2.35b	14.5 ± 2.34b	9.56 ± 0.96b	0.75 ± 0.09b	0.68 ± 0.86b	0.33 ± 0.17b
20	104 ± 4.32a	122 ± 15.9a	95.7 ± 20.4a	12.1 ± 1.61bc	11.1 ± 2.41bc	4.68 ± 1.86b	0.70 ± 0.06b	0.33 ± 0.03b	0.17 ± 0.08b
40	76.1 ± 4.79a	91.6 ± 16.3a	52.6 ± 4.58ab	7.49 ± 1.24bc	7.78 ± 2.16bc	1.63 ± 0.52b	0.33 ± 0.02c	0.35 ± 0.09b	0.17 ± 0.05b
80	75.7 ± 17.1a	52.0 ± 1.81b	N/A	4.11 ± 0.78c	3.25 ± 0.82c	N/A	0.22 ± 0.04c	0.07 ± 0.01b	N/A

[1] Data represent the mean ±1 SE (n = 3). Means in a column followed by the same letter are not significantly different (Tukey test, $p \leq 0.05$).

Chlorophyll concentrations 7 DAT indicated that higher rates of fluridone produced more severe symptoms of plant injury than lower rates (Table 1) as seen in other CET studies (Netherland et al. 1993, Netherland and Getsinger 1995a, 1995b). At 30 DAT, chlorophyll concentrations decreased for all treatments, including the 10 µg ai L^{-1} rate. These low chlorophyll concentrations were further evidence that the plants stopped growing and were starting to decay.

AVAST! was successful in virtually eliminating milfoil at the rates tested in this study, 10, 20, 40, and 80 µg ai L^{-1}. At these rates, a 45-day exposure time was sufficient to reduce 90 percent of plant biomass. Symptoms of plant injury were evident 7 DAT with significant differences between rates; however, by 45 DAT, these differences were obscured as concentrations of chlorophyll, phytoene, and β-carotene were the same for all treatments, except the reference.

3 Selectivity Study

Methods and Materials

This mid-scale study was conducted from April through July 2000 in 15 outdoor mesocosms (1.4 m tall by 2.6 m in diameter, volume = 7000 L, water depth = 130 cm) located at the U.S. Army Engineer Research and Development Center, Lewisville Aquatic Ecosystem Research Facility (LAERF), Lewisville, TX. A description of the mesocosm system is summarized in Dick et al. (1997).

Plastic containers (19.7 cm tall by 19.7 cm in diameter, volume = 5 L) were filled with sediment collected from a pond located at the LAERF. The LAERF pond sediment characteristics are summarized in Smart et al. (1995). The sediment in each container was amended with 10 g ammonium sulfate (21-0-0) and 1 "Woodace" briquette (14-3-3).

Four submersed aquatic plants usually found in association with Eurasian watermilfoil in northern tier states represented nontarget species (Nichols and Buchan 1997): wild celery (*Vallisneria americana* Michx.), elodea (*Elodea canadensis* Michx.), sago pondweed (*Stukenia pectinata* (L.) Borner), and Illinois pondweed (*Potamogeton illinoensis* Morong). Milfoil, elodea, and Illinois pondweed were gathered from LAERF pond cultures and rooted using three apical stems (15 to 20 cm) per container. Three tubers of sago pondweed (Kester's Wild Game Food Nurseries, Omro, WI) were propagated in each container. Wild celery (Suwannee Laboratories, Lake City, FL) was propagated using two small plants (shoots + roots = 15 to 30 cm) per container. Each mesocosm was divided into five sections to accommodate five pots of each plant species. These plants were planted 3 through 7 April and allowed to establish for 42 days before herbicide application.

On 16 May, the aqueous suspension of AVAST! was applied to mesocosms in doses of 6, 12, and 24 μg ai L^{-1} for a static exposure of 56 days. Untreated references compared plant growth and physiology in the absence of herbicide dosage. Timing of application coincided with peak biomass and low carbohydrate storage reported for milfoil growing in the LAERF ponds (Madsen 1997).

Water temperature, pH, alkalinity, and turbidity were measured in the mesocosms during the evaluation period.

Water samples were collected in 500-mL amber high-density polyethlene bottles at 1, 7, 14, 21, 28, 35, 42, and 49 DAT to monitor herbicide degradation in the water column. Samples were analyzed using high performance liquid chromatography (HPLC). This method has been employed by Getsinger et al. (2001) and Netherland et al. (2002). All water residue analytical procedures were conducted using a Waters HPLC system, made up of the following components: Waters 510 delivery pump, Waters 486 UV detector, Waters 746 data integrator, and incorporating a Waters μ Bondapak C18, 3.9 x 300 mm HPLC column. Solid phase extraction (SPE) cartridges were used to clean the water samples as well as concentrating fluridone. The Waters SPE-Pak Vac 6 cc (500 mg) C18 cartridges were placed on a 12-place SPE-Pak vacuum manifold (JT Baker PN 7018-00). After column conditioning procedures, a 100-mL water sample was filtered through the SPE cartridges and eluted with 2 mL methanol. Samples were collected and stored in 4-mL amber glass vials and held until analysis. Fluridone concentrations in water were determined by comparison of the detector response by peak area for the samples against the peak area response obtained from known standard concentrations of fluridone. Standards were prepared from analytical grade fluridone (99.1 percent purity) obtained from Griffin LLC. The HPLC conditions were set as follows: eluent for mobile phase was 65:35 methanol: water; chart speed was set at 0.25 cm min^{-1}; flow rate was 1.2 mL min^{-1}; wavelength was 313 nm, attenuation was 8 because standard values were set at 0.2 mg L^{-1}; and the sample injection volume was 100 μL. Run time for this compound was approximately 10 min, with the fluridone peak registering at 7 min. The reporting limit for this method is 1.0 μg L^{-1}. A series of blind sample spikes were analyzed with a mean percent recovery of 90 percent (63 to 118 percent).

Apical plant stems (3 to 5 cm) were collected from each treatment at 28 and 56 DAT, 15 June and 14 July, respectively. Plant tissue samples were analyzed for the pigments phytoene and β-carotene after Sprecher et al. (1998), and total chlorophyll content after Hiscox and Israelstam (1979) using 10 ml of DMSO for extraction.

To assess efficacy of fluridone exposure, shoots were harvested from four containers of each species at 56 DAT, 14 July. Shoots were cut at the sediment surface, oven dried at 70 °C for 48 hr, and then weighed.

Treatments were randomly assigned to each mesocosm and replicated three times. Herbicide residue data were regressed against time using the exponential decay model:

$$y = a * \exp(-b*x)$$

where

y = chemical concentration

a = intercept of regression line

b = slope of regression line

x = sampling time

Dissipation half-lives ($t_{½}$) of fluridone were then calculated using the slope (b) of each significant regression ($p < 0.05$) in the equation:

$$t_{½} = -\ln(0.5)/b$$

Biomass data were transformed using the square root function to satisfy the assumptions of normality and equal variance, then subjected to a one-way ANOVA to test for herbicide concentration effects. If pigment data did not meet the assumptions of normality and equal variance, data were log transformed, then analyzed using a one-way ANOVA to test for herbicide concentration effects at each sample date. If significant differences were detected for each ANOVA, means were separated using the Tukey test ($p \leq 0.05$).

Results and Discussion

Water temperatures in the mesocosms were 27 °C at the time of herbicide application, and ranged from 25 to 32 °C during the study, 16 May to 14 July. Alkalinity decreased from 36 ± 0.9 at the time of application to 28 ± 1.6 meq L^{-1} at the end of the study. The pH increased from 8.4 ± 0.2 to 9.1 ± 0.2. Turbidity was very low throughout the study (0.4 to 1.3 NTU).

Water residues measured at 1 DAT matched target concentrations (Table 2), except for the 24 µg ai L^{-1} treatment, which was was 13 percent higher than the target concentration (27.9 ± 1.23 µg ai L^{-1}). Target concentrations were maintained through 7 DAT. By 14 DAT, fluridone was gradually dissipating from the water column with concentrations 45 to 50 percent below the target rates (Table 2). Half-lives for all treatments ranged from 23.1 to 24.7 days (Figures 3-5). These residues indicated that threshold fluridone concentrations required to effectively control milfoil remained in the mesocosms until the end of the 56-day exposure period (Netherland and Getsinger 1995a).

Table 2
Water Residues (µg ai L^{-1}) in Outdoor Mesocosms Following Application of the AVAST! Formulation of Fluridone, May through July 2000[1]

Target Application Rate µg ai L^{-1}	Concentration of Fluridone in Water Residues, µg ai L^{-1}							
	1 DAT	7 DAT	14 DAT	21 DAT	28 DAT	35 DAT	42 DAT	49 DAT
6	6.59 ± 0.21	6.05 ± 0.36	2.73 ± 0.59	3.38 ± 0.12	2.75 ± 0.61	2.41 ± 0.27	2.29 ± 0.34	2.32 ± 0.43
12	13.5 ± 0.63	11.3 ± 1.30	6.15 ± 1.31	7.93 ± 0.93	4.75 ± 0.55	5.06 ± 1.33	4.60 ± 0.68	4.01 ± 0.22
24	27.9 ± 1.23	24.5 ± 0.62	12.6 ± 2.84	14.4 ± 0.54	NS[2]	10.5 ± 0.65	9.79 ± 1.59	8.42 ± 0.58

[1] Residues analyzed by HPLC. Data represent the mean ±1 SE (n = 3).
[2] NS=No sample taken.

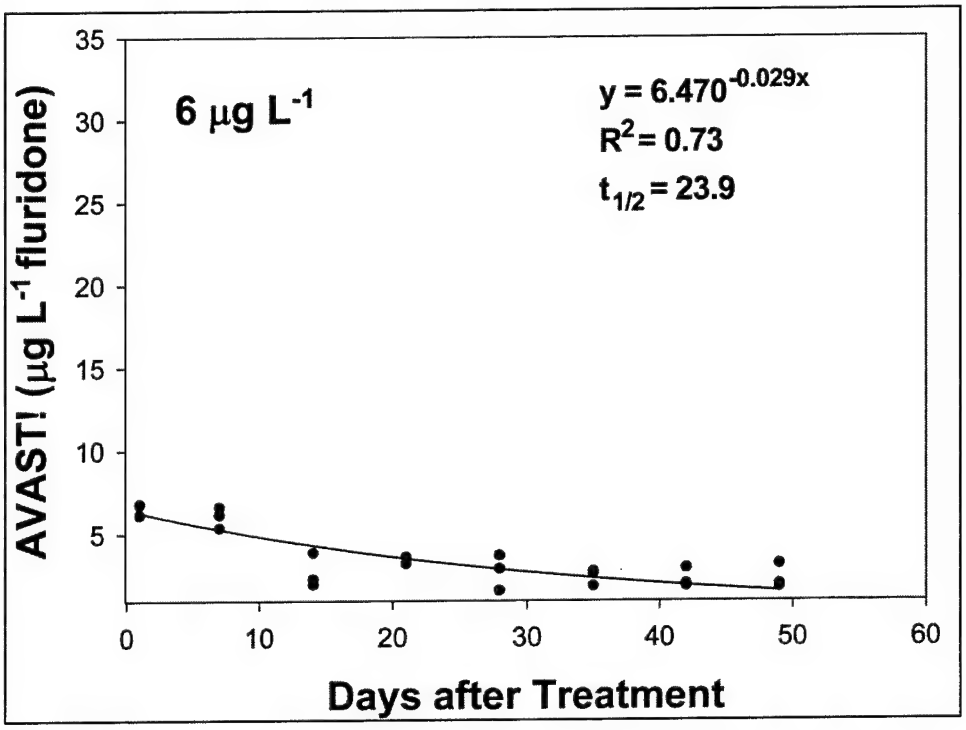

Figure 3. Dissipation and half-life in outdoor mesocoms treated with 6 µg ai L^{-1} of the AVAST! fluridone formulation (n = 24), May through July 2000

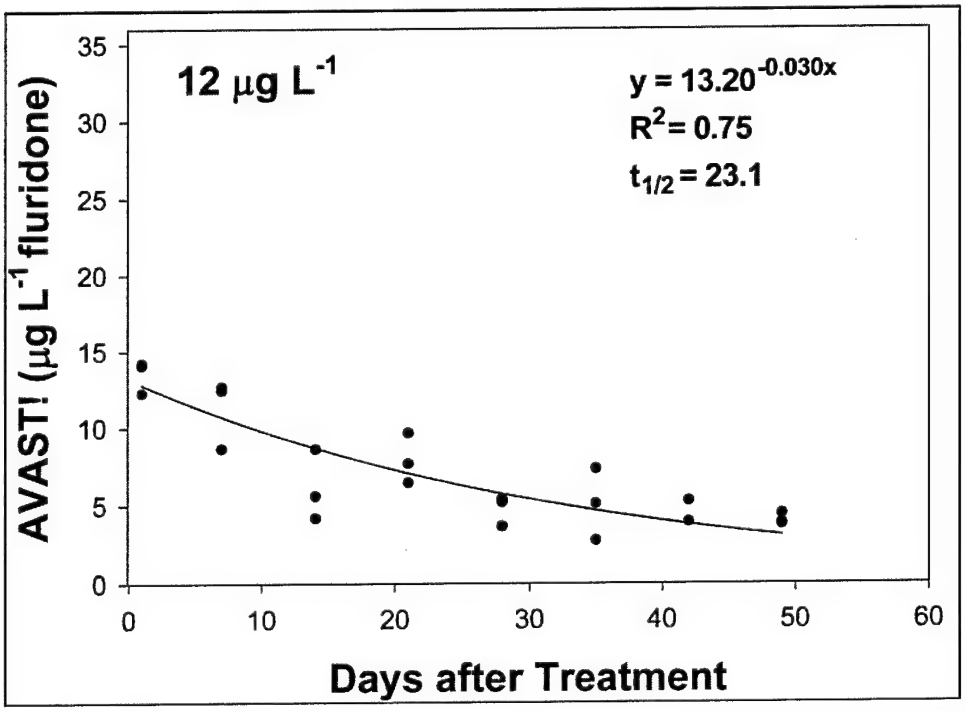

Figure 4. Dissipation and half-life in outdoor mesocoms treated with 12 µg ai L^{-1} of the AVAST! fluridone formulation (n = 24), May through July 2000

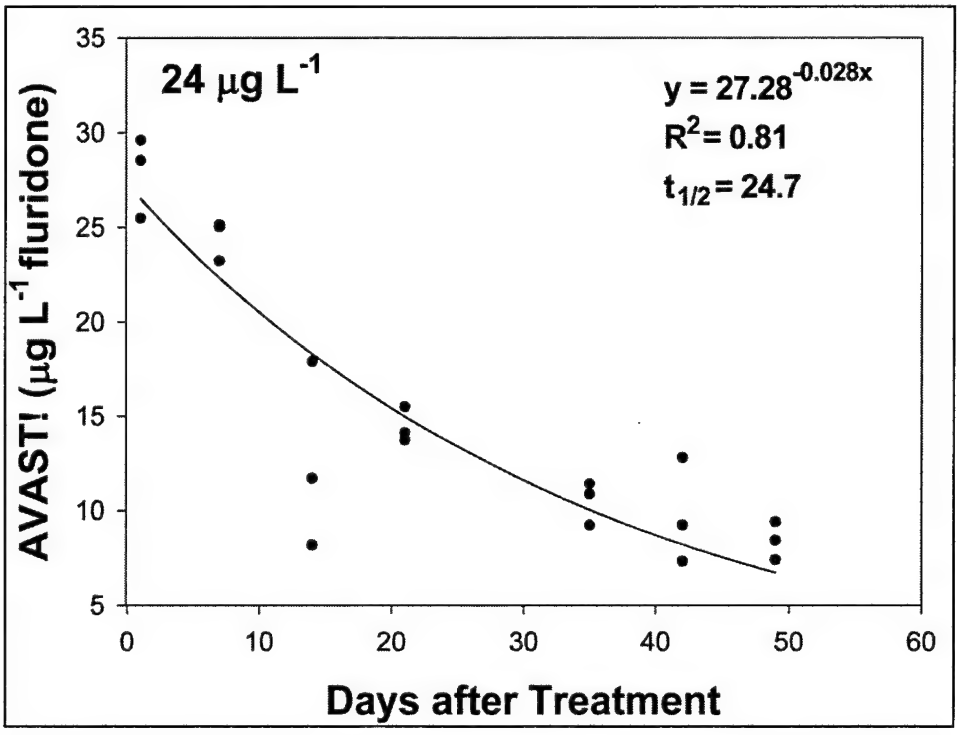

Figure 5. Dissipation and half-life in outdoor mesocoms treated with 24 µg ai L^{-1} of the AVAST! fluridone formulation (n = 21), May through July 2000

As a result, milfoil shoot biomass decreased by 90 percent for all AVAST! treatments (Figure 6). No significant differences were detected between application rates. These results mirror those found in the CET chamber study where milfoil was controlled by 90 percent after 45 days and 99 percent after 90 days for AVAST! treatment rates ranging from 10 to 80 µg ai L^{-1} (Figure 2). Similar results have been reported by other researchers that conducted mesocosm evaluations at the LAERF using the Sonar® AS fluridone formulation. Netherland et al. (1997) described how a May application of Sonar controlled milfoil by 90 percent for rates ranging from 5 to 20 µg ai L^{-1} after a 60-day exposure period. Nelson et al. (1998) reported a 75 percent reduction in shoot biomass by 5 µg ai L^{-1} of Sonar.

Increases in phytoene pigment concentrations concomitant with decreases in β-carotene and chlorophyll levels reflect the efficacy of fluridone against milfoil (Table 3). Although efficacy was similar, trends in these pigment concentrations were more pronounced in this mesocosm study than in the indoor growth chamber study (Table 1), probably due to the high light conditions outdoors (Bartels and Watson 1978, Doong et al. 1993). Because milfoil shoot tips were necrotic, no tissue samples were available for analysis 56 DAT. Nonetheless, β-carotene and chlorophyll levels at 28 DAT for the 6 and 12 µg ai L^{-1} treatments suggested that plants may recover from fluridone exposure. Milfoil recovered when placed in untreated fresh water in one mesocosm study (Netherland et al. 1997), and chlorophyll concentrations of 0.98 µg g FW^{-1} in remaining shoots indicated potential recovery in another mesocosm study (Nelson et al. 1998). Field studies have corroborated these small-scale results in which adequate

exposure time is critical to maintaining control of milfoil (Getsinger et al. 2001, 2002a, Madsen et al. 2002).

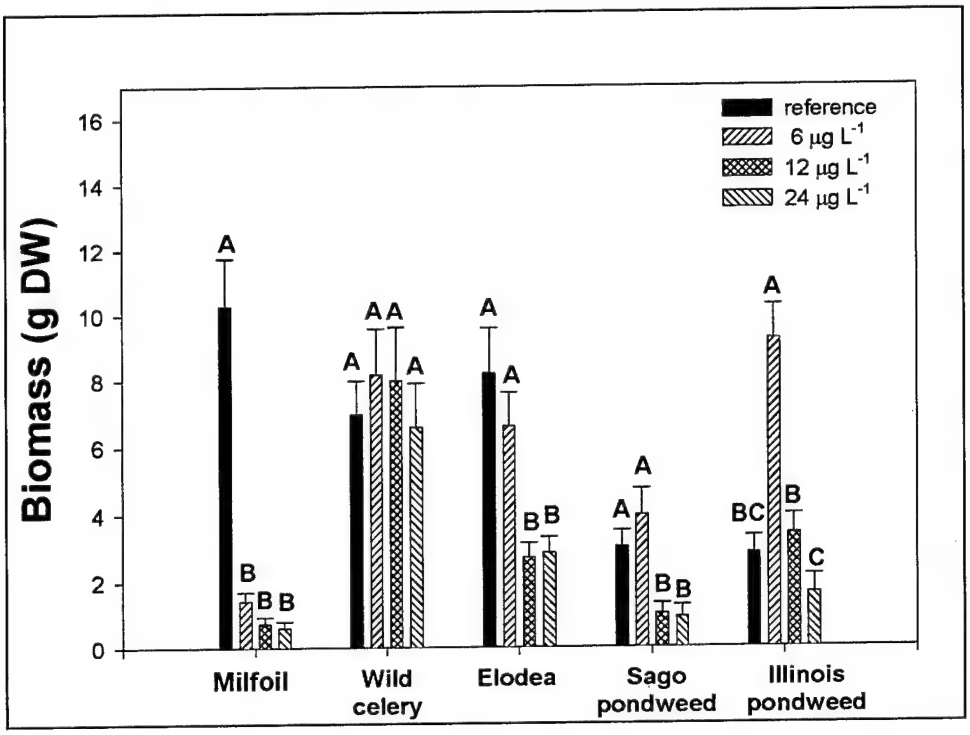

Figure 6. Shoot biomass (g DW) of aquatic plants harvested after 56-day exposure to the AVAST! formulation of fluridone. Rates tested were 6, 12, and 24 µg ai L^{-1}. Means are ±1 SE (n = 3). Letters denote significant differences between treatments for each species (Tukey test, $p \leq 0.05$)

Response of nontarget plants to fluridone applications varied with species and application rate as in previous mesocosm studies that evaluated Sonar® AS (Netherland et al. 1997, Nelson et al. 1998). Although there were no significant shoot biomass reductions between the reference and treated plants in wild celery and Illinois pondweed, significant reductions were observed with higher application rates in elodea and sago pondweed (Figure 6).

Elodea is considered highly susceptible to fluridone (Westerdahl and Getsinger 1988). In this study, elodea shoot biomass was reduced by >70 percent with 12 and 24 µg ai L^{-1}, but no significant reduction occurred with 6 µg ai L^{-1} (Figure 6). Pigment concentrations support these results (Table 3). Phytene levels of >200 µg g FW^{-1} signified the uptake of fluridone in all plants, while β-carotene and chlorophyll levels indicated the degree of plant injury. In contrast, elodea was not injured with rates as high as 20 µg ai L^{-1} from a May application of fluridone in another mesocosm study (Netherland et al. 1997). However, elodea biomass and distribution has reportedly declined following field applications using rates as low as 5 µg ai L^{-1} (Getsinger et al. 2001, 2002b).

Like elodea, sago pondweed shoot biomass was reduced by >70 percent with 12 and 24 μg ai L^{-1}, but no significant reduction occurred with 6 μg ai L^{-1} (Figure 6). Similar results have been reported in other small-scale studies (Netherland et al. 1997, Sprecher et al. 1998). Because sago pondweed frequency has both increased (Smith and Pullman 1997, Getsinger et al. 2001) and declined (Getsinger et al. 2002b) after field applications, plant survival might be attributed to factors other than herbicide exposure, such as timing of herbicide application relative to phenology (Smith and Pullman 1997), natural shifts in community assemblages (Madsen et al. 2002), and presence of inflow areas that create refugia (Smith and Pullman 1997). Based on pigment concentrations, recovery of sago pondweed from exposure to 12 μg ai L^{-1} fluridone in this study was possible. By 56 DAT, phytoene concentrations decreased and β-carotene concentrations increased in these plants, suggesting that sago pondweed shoots may have stopped taking up the herbicide and started to generate more carotenoid pigments (Table 3), thereby increasing its photosynthetic capability.

Although elevated phyotene concentrations confirmed that fluridone was taken up by wild celery shoots (Table 3), biomass was not significantly affected by herbicide exposure at any rate tested (Figure 6). Levels of β-carotene, which were similar to the reference by 56 DAT, reflect growth and survival of this species (Table 3). Wild celery also survived when treated with 5 μg ai L^{-1} fluridone in another mesocosm study (Nelson et al. 1997), but was greatly reduced when treated with higher rates (Netherland et al. 1997, Sprecher et al. 1998). Field results vary for this species (Smith and Pullman 1997, Getsinger et al. 2001, 2002b).

Illinois pondweed, like wild celery, was not adversely affected by fluridone exposure (Figure 6), although relatively high phytoene concentrations showed that the plants were taking up the herbicide (Table 3). Although no data are available for 56 DAT, β-carotene levels were similar for all plants, including the reference (Table 3). These results are supported by the AVAST! label, which states that Illinois pondweed is partially controlled by the AVAST! formulation of fluridone. Smith and Pullman (1997) also described Illinois pondweed as intermediately sensitive to fluridone, which has been verified in field applications (Getsinger et al. 2001, 2002b).

AVAST! successfully controlled milfoil by 90 percent for all rates tested in this study, 6, 12, and 24 μg ai L^{-1} during a 56-day exposure period. Gradual herbicide dissipation in the mesocosms resulted in half-lives that ranged from 23.1 to 24.7 days. AVAST! did not adversely affect the nontarget species wild celery and Illinois pondweed; however, shoot biomass reductions were observed with application rates of 12 and 24 μg ai L^{-1} in elodea and sago pondweed. Symptoms of injury were evident in these plants by depressed β-carotene pigment concentrations at 28 DAT; however, β-carotene levels increased in sago pondweed by 56 DAT, suggesting recovery.

Table 3
Pigment Concentrations in Shoots of Milfoil and Nontarget Plants 28 and 56 DAT with AVAST! Fluridone Formulation in an Outdoor Mesocosm Study, May through July 2000[1]

Fluridone µg ai L^{-1}	Phytoene, µg g FW^{-1}		β-Carotene, µg g FW^{-1}		Chlorophyll, mg g FW^{-1}	
	28 DAT	56 DAT	28 DAT	56 DAT	28 DAT	56 DAT
Milfoil[2]						
0	16.2 ± 0.63 b		29.0 ± 1.59 a		1.08 ± 0.10 a	
6	115 ± 10.4 a		10.6 ± 1.53 b		0.73 ± 0.12 ab	
12	106 ± 12.4 a		15.0 ± 4.58 b		0.46 ± 0.13 bc	
24	45.7 ± 3.46 b		5.03 ± 0.88 b		0.23 ± 0.06 c	
Wild Celery						
0	10.8 ± 1.13 b	14.6 ± 0.86 b	25.6 ± 3.5 a	24.4 ± 7.00 a	0.73 ± 0.23 a	0.77 ± 0.20 a
6	74.0 ± 15.2 ab	38.4 ± 3.56 ab	15.5 ± 1.72 ab	17.9 ± 0.52 a	0.50 ± 0.02 a	0.50 ± 0.08 a
12	92.3 ± 20.4 a	82.5 ± 27.9 a	9.84 ± 3.33 b	15.6 ± 4.47 a	0.51 ± 0.03 a	0.42 ± 0.04 a
24	99.4 ± 22.9 a	94.1 ± 0.95 a	4.93 ± 0.78 b	5.95 ± 2.78 a	0.42 ± 0.05 a	0.36 ± 0.11 a
Elodea						
0	16.5 ± 0.55 b	17.2 ± 1.46 b	32.6 ± 2.90 a	35.2 ± 2.06 a	1.20 ± 0.27 a	1.05 ± 0.02 a
6	231 ± 31.5 a	98.0 ± 43.6 ab	15.6 ± 1.49 b	22.5 ± 1.36 b	0.67 ± 0.11 ab	1.00 ± 0.24 a
12	245 ± 32.3 a	209 ± 55.9 ab	9.21 ± 1.32 bc	15.8 ± 1.38 bc	0.25 ± 0.04 b	0.36 ± 0.09 a
24	227 ± 11.1 a	263 ± 56.9 a	6.01 ± 0.51 c	12.5 ± 2.51 c	0.23 ± 0.02 b	0.44 ± 0.08 a
Sago Pondweed						
0	17.7 ± 1.75 b	14.1 ± 2.70 a	19.0 ± 1.14 a	17.2 ± 3.03 a	0.85 ± 0.05 a	0.79 ± 0.15 a
6	115 ± 21.3 a	28.8 ± 17.3 a	16.0 ± 3.13 ab	16.3 ± 2.95 a	0.83 ± 0.06 a	0.62 ± 0.21 a
12	109 ± 29.0 a	41.8 ± 2.25 a	7.34 ± 1.75 bc	9.24 ± 1.74 a	0.44 ± 0.1 a	0.33 ± 0.09 a
24	67.6 ± 14.5 ab		6.22 ± 0.62 c		0.18 ± 0.02 a	
Illinois Pondweed[2]						
0	21.2 ± 2.80 b		32.7 ± 5.37 a		1.01 ± 0.21 a	
6	168 ± 6.69 ab		28.9 ± 10.4 a		1.00 ± 0.27 a	
12	191 ± 14.8 a		13.7 ± 2.02 a		0.50 ± 0.09 a	
24	96.4 ± 34.5 ab		8.09 ± 1.43 a		0.23 ± 0.06 a	

[1] Data represent the mean ±1 SE (n = 3). Means in a column followed by the same letter are not significantly different for each species (Tukey test, $p \leq 0.05$).
[2] No plant material available for sampling for milfoil and Illinois pondweed at 56 DAT or sago pondweed treated with 20 µg ai L^{-1} at 56 DAT.

4 Conclusions

Based on the results of these small- and mid-scale studies, the following conclusions can be drawn:

a. The AVAST! aqueous suspension formulation of fluridone is very effective against the target plant Eurasian watermilfoil. Rates as low as 6 µg ai L^{-1} provided >90 percent reduction in shoot biomass after a 60-day exposure period, in which the herbicide dissipation half-life was 23.9 days. Higher rates (10 to 80 µg ai L^{-1}) provided up to 99 percent control.

b. Based on pigment concentrations, milfoil may have recovered if allowed to grow in untreated fresh water following the fluridone exposure periods; however, continued herbicide exposure would have completely controlled milfoil as lethal threshold fluridone concentrations were still present in the mid-scale mesocosm evaluation after 49 days.

c. Nontarget submersed plants typically susceptible to fluridone were injured by AVAST! at rates greater than 6 µg ai L^{-1}, including elodea and sago pondweed. Pigment concentrations suggested that sago pondweed may recover following removal of fluridone from surrounding water. Wild celery and Illinois pondweed were not adversely affected at any rate of AVAST! tested.

d. Previously developed physiological markers, plant pigment concentrations of total chlorophyll, phytoene, and β-carotene, can be used to monitor injury of plant exposed to AVAST!.

Recommendations

Based on the results of these small- and mid-scale studies, the following recommendations are provided:

a. Additional mesocosm studies should be conducted to more accurately determine the potential of fluridone to selectively control Eurasian watermilfoil at rates between 5 and 10 µg ai L^{-1}. These evaluations would expand on the growth and survival of ecologically important nontarget submersed species.

b. Results of these mesocom studies should be verified by large-scale whole-lake demonstrations in water bodies infested with milfoil.

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14. ABSTRACT

Experiments were conducted in a laboratory and an outdoor mesocosm system to evaluate the liquid AVAST!® fluridone formulation for control of Eurasian watermilfoil (*Myriophyllum spicatum*). In addition, physiological assays were used to monitor plant injury during herbicide exposure. Eurasian watermilfoil was planted in 52-L aquaria, grown to precanopy condition (21 days), then dosed with 0, 10, 20, 40, and 80 µg L^{-1} active ingredient (ai) fluridone for exposure times of 45 and 90 days. Elevated phytoene concentrations indicated herbicide exposure in all treated plants at 7 days after treatment (DAT). Visually, plants did not manifest symptoms of injury from fluridone until 14 DAT. β-carotene concentrations suggested that fluridone disrupted photosynthesis as soon as 7 DAT. Both formulations were effective in controlling Eurasian watermilfoil. Biomass decreased by 90 percent at all application rates following the 45-day exposure and decreased by 99 percent following the 90-day exposure time. No significant differences occurred between application rates at either exposure time. Based on these results, rates of 6, 12, and 24 µg ai L^{-1} fluridone were applied to Eurasian watermilfoil and four nontarget submersed aquatic species in an outdoor mesocosm system for a 56-day exposure time. Gradual herbicide dissipation in the mesocosms resulted in half-lives ranging from 23 to 24 days. Again, the AVAST!® fluridone formulation was effective in controlling Eurasian watermilfoil. Biomass was reduced by >85 percent at all doses compared to the untreated reference. The AVAST! formulation did not significantly decrease biomass from wild celery (*Vallisneria americana*) and Illinois pondweed (*Potamogeton illinoensis*) at any application rate. Biomass levels from elodea (*Elodea canadensis*) and sago pondweed (*Stuckenia pectinata*) were reduced at 12 and 24µg ai L^{-1}. Symptoms of injury were evident in these plants by depressed β-carotene pigment concentrations at 28 DAT; however, β-carotene levels increased in sago pondweed by 56 DAT, suggesting recovery.

15. SUBJECT TERMS

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15. SUBJECT TERMS

B-carotene
Chemical control
Elodea canadensis
Eurasian watermilfoil
Herbicide
Illinois pondweed
Myriophyllum spicatum
Phyotene
Potamogeton illinoensis
Sago pondweed
Stuckenia pectinata
Vallisneria americana
Wild celery